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Biocompatibility of polyimide-based neural interfaces for chronic implant applications

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Neural interfaces provide functional re-establishment of the central nervous system, as well as accessibility to monitor physiological responses at a cellular and molecular level. An ideal device is electrically, mechanically, and biologically compatible in long-term applications. Reducing the foreign body response and scar tissue formation caused by the surrounding tissue of the implant makes the device most biocompatible, while not hindering the electrical interface of the device. Technological advances in polymer materials are leading to improved designs of neural implants with the utilization of flexible polyimide, which decreases the relative micromotion strain. However, the flexibility of polyimide causes the device to buckle during insertion; therefore a biodegradable polymer, polyglycolic acid (PGA), is added to the polyimide device in order to temporarily enhance the structural rigidity. In this study we detail the successful biocompatibility demonstrated with both uncoated and PGA coated polyimide devices to provide new design strategies for neural implants used in chronic applications.

Introduction

An enormous challenge in neuro prosthesis is designing an electrode device which can be dependable, stable, and easily implanted with minimal damage. Neural interfaces serve numerous purposes, such as aiding in the functional restoration of the central nervous system and as signal recording devices to understand the electrophysiological environment at a cellular and molecular level. Continuous advancements in polymer materials lead closer to the design of an ideally biocompatible neural device for long-term situations.

Early devices consisted of a stiff, insulated metal wire which could be inserted with no buckling. Without repositioning, signal recordings of a neuron lasted for only several hours in optimal conditions; therefore, this material lost appeal in the design of chronic implants. Further material advancements led to silicon-based electrodes which provided a batch fabrication appeal for increased device assembly reproducibility. Silicon was still penetrating cortical tissue upon insertion, as were the previously described devices. However, as with the previous electrodes, silicon-based devices lacked the capability for long-term implant applications being as their recording capabilities diminished with time.¹ Most recently, polymer-based devices were being considered for chronic implant designs. Devices fabricated with polyimide were appealing because of material's dielectric properties, mechanical flexibility, biocompatibility, and batch fabrication. In earlier investigations by Stieglitz *et al.*,² polyimide was found to be reliable in physiological environments as it did not degrade with time and was non-toxic to surrounding cells. Furthermore, polyimide was light weight, conquering the mechanic challenges of the previously considered materials, making it a more ideal choice.²

A key appeal of polyimide was the mechanical flexibility it demonstrated; neither silicon nor microwire used in previous neural interfaces exhibited this characteristic. In these previous cases, micromotion between the electrode device and the soft biological tissue was mismatched, leading to inflammation and tissue scarring. Resulting tissue damage and biological response led to isolation of the device's electrical interface from the tissue it was targeting to signal. Researchers hypothesized the increased mechanical compliance may aid to minimize the tissue's foreign body response. Polyimide significantly reduced the mechanical impedance mismatch being as tungsten microwire had a Young's modulus of 400 *GPa*, silicon had a Young's modulus of approximately 170 *GPa*, polyimide had a Young's modulus of approximately 3 *GPa*, and brain tissue had a Young's modulus of 0.067 *GPa*.³⁻⁵ Although polyimide's mechanical flexibility made it an attractive material to utilize, it buckled during surgical insertion. Furthermore, this implant procedure required the removal of dura and pia, disrupting connective tissue layers lying above the brain, and thus causing greater tissue damage. To enhance the mechanical rigidity of the device for tissue insertion we apply PGA, Young's modulus of approximately 7 *GPa*, onto the device interface. PGA has glass transition temperature of 35° *C*, allowing the material to be stable at room temperatures and soften after insertion. As the material undergoes glass transition, it was then biodegraded within several days after implantation. PGA is an established, FDA approved, biodegradable material commonly used in sutures, which was utilized to develop polyimide devices.^{5,6}

In addition to mechanical compatibility, the device materials had to enhance biocompatibility by producing a reduced immune response in surrounding neural tissue, decreasing the risk of tissue scarring and device

isolation. Although gliosis, the accumulation of glial cells where neurons have been damaged, was a concern when introducing the electrode into neural tissue, both polyimide and PGA were shown to be compatible in neurobiological-environments. To further increase the biocompatibility of polyimide, a nerve growth factor could be incorporated into these devices, increasing cell adhesion interactions.^{1,5}

Using histology methods and material analyses, we show the compatibility of uncoated and PGA coated polyimide-based devices for neural interfaces in chronic implant situations. Understanding mechanical and biological compatibilities of polymer materials provide new insights and strategies for neural electrode designs of long-term implants.

Materials and methods

PGA degradation

Small pieces (25 – 35 mg) of PGA were submersed in 15 ml of 0.9% saline solution. Weight measurements of PGA were taken before submersion, along with *pH* measurements of the saline solution. After one day, the previous saline solution was replaced with fresh solution once the remaining PGA had been weighed. The weight measurements continued at 1 day intervals until the PGA was completely degraded. Along with recording weight, measurements of *pH* were also taken at specific intervals. The solutions were incubated at 37° C when measurements were not being taken. In comparison to in vivo material experiments, the saline solution reflects similar salinity and electric properties of the physiological environment. Material testing methods in vitro include the use of a cell culture medium, using a saline-based solution; thus the saline tests prove to be an adequate and simpler technique for PGA analysis.

Electrode fabrication

Polyimide-based electrodes were fabricated from a previously established protocol.⁶ In order to create the devices, a silicon wafer was initially used to create the foundation of the devices. The devices were constructed without gold traces, as the metal was utilized only for electrical recording purposes. Figure 1 details the electrode geometric dimensions of the produced device, resulting in shafts 4 mm lengths $\times$ 146–156 μ m widths. In addition, some electrodes were melt-coated with PGA, increasing the geometric thickness of the device. The melt-coating technique consisted of first melting the PGA, keeping the material temperature below the denaturation temperature. The polyimide devices were then swiped through the PGA, allowing the PGA to coat the devices on only one side. This method consequently created a partial coating on the reverse side of the device. Melt-coating

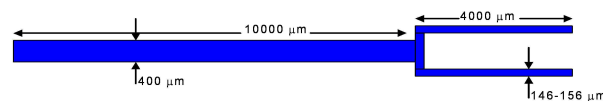


FIG. 1: Schematic drawing of the final dimensions and geometry of polyimide electrode devices. Devices such as these were implanted into the central nervous tissue to assess biocompatibility.

produced devices with an approximate ratio of 10 : 1, PGA:polyimide. Devices were developed using photo-definable polyimide in the Nanofabrication Core Facility at the University of Illinois at Chicago.

Surgical procedure

All animal procedures were performed in agreement with the University of Illinois at Chicago Animal Care Committee guidelines. Prior to surgery, male Sprague-Dawley rats (300–350 g body weight) were anaesthetized with an intramuscular injection. Anesthesia consisted of ketamine, xylazine, and acepromazine (KXA). Supplemental doses of KXA were given to maintain a constant depth of anesthesia during surgery, monitored through paw-pinch reflexes, pulse rate, and oxygen saturation.

The chronic surgery required the use of sterile equipment and tools for procedures. Polyimide device sterilization constituted submerging the devices in 70% isopropyl alcohol for 1 hour, and then storing under sterile conditions until used. The animal's skull was positioned in a stereotaxi frame before beginning surgical procedures. Surgical procedures began with craniectomies for the placement of a stainless steel bone screw and three polyimide devices. Polyimide electrodes were hand-implanted into each subject.

Histology procedures

Fifteen days post-implant, animals were perfused for tissue preparation and fixation. Animals were first given a large dosage of KXA anesthesia, followed by perfusion of 0.9% saline solution, and then 10% formalin solution. The brain tissue was extracted and stored in formalin fixative. The whole brain tissue was divided to separate the three implant areas. The tissue was dehydrated and fixed with the following procedure: 70% ethanol (> 4 hours), 95% ethanol (4 hours), 100% n-butanol (2 $\times$ 12 hours), equal parts of n-butanol and toluene (12 hours), toluene (12 hours), paraffin (24 hours).

Paraffin embedded tissue was serially sectioned at an 8 μ m thickness, cutting perpendicular to the device. Sections were collected to an implant depth of 2-3 mm to obtain a significant amount of data for qualitative analysis. Free floating sections were mounted for hematoxylin and eosin (H&E) and immunohistochemistry staining. H&E stains were performed in approximate depth intervals at

every 250 μm ; immunohistochemistry stains were performed in approximate depth intervals at every 500 μm .

Immunohistochemistry staining was done by first deparaffinizing mounted sections in xylene, followed by a hydration procedure carried out through a series of alcohol dilutions. Next, an antigen retrieval process was used, which included incubating the slides in 0.1 M citrate buffer ($\text{pH} = 6.0$) at 95–100° C. Sections were processed using a primary polyclonal rabbit antibody targeting glial fibrillary acidic protein (GFAP) (DakoCytomation Cat# Z0334), a secondary biotinylated goat anti-rabbit antibody, a Vectastain Elite ABC reagent (Vector Laboratories, Inc.), and finally a diaminobenzidine solution containing nickel intensification (Vector Laboratories, Inc.). Slides, including both H&E stains and immunohistochemistry stains, were imaged with brightfield microscopy and used for qualitative analysis.

Results

Histological assessments, used to examine the tissue immune responses, were coupled with material analyses to determine the biocompatibility of polyimide and PGA used in neural interface designs.

PGA mass and pH measurements for material degradation

Both mass degradation and pH analyses were conducted to assess the biocompatibilities of PGA. Both measurements were evaluated over specific time intervals. The mass degradation profile in Figure 2(a) shows the time period required for this amount of PGA to degrade. Material mass (25 – 35 mg) is shown to completely degrade within several days. Figure 2(b) displays the pH profile of saline solution with 25 – 35 mg pieces of PGA material. The solution becomes slightly acidic, however this is a minimal affect.

Cell behavior with H&E histology

Tissue samples analyzed with an H&E staining procedure were used to assess the cell behavior of tissue surrounding the implant. Abnormal cell distribution would be characterized by increased cell density around the device implant, as a foreign body response. Figure 3 displays the typical cell-material interaction we found between the electrode and surrounding tissue. Qualitative analysis shows minimum tissue response to the implanted devices, as the cell density isn't increased enough to create a barrier which would hinder the electrical recording capabilities. Figure 3(a)-3(c) displays tissue sections of three uncoated polyimide devices. Figure 3(d)-3(f) shows tissue sections at various depths of three PGA coated polyimide implants. The images show a slight

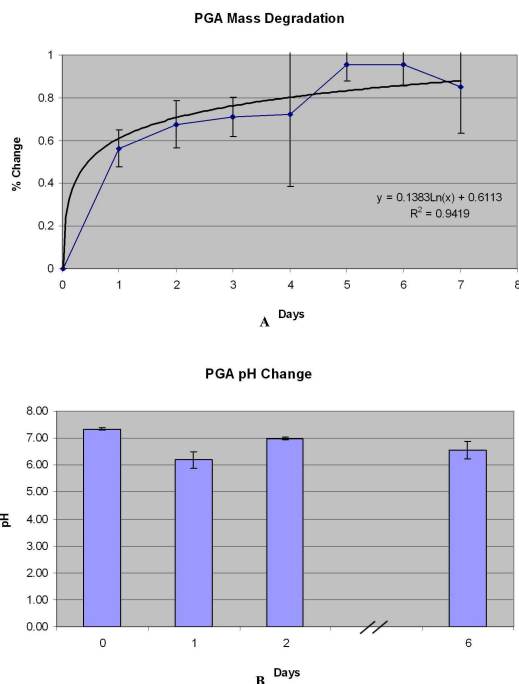


FIG. 2: Assessment of the biocompatibility of PGA material. pH measurements ($n=5$) were documented, along with mass degradation measurements ($n=5$). Error bars represent standard deviation from the data population. a) PGA material was weighed during solution changes at 1 day interval to assess the time needed for the materials to degrade. The mass change was computed until the material was completely degraded. b) Measurements of pH were taken and documented before the saline solution change for this particular day.

one-sided response around the PGA coated devices, observed through increased cell density on one side of the device. The images were taken of sections at varying implant depths to thoroughly characterize the cell-material interactions surrounding the device.

Immune response analysis with GFAP immunohistochemistry

GFAP is a major component of astrocytes and therefore commonly used as an astrocyte identification marker for tissue of the central nervous system.³ Figure 4 shows the typical immunohistochemistry results of implanted tissue samples found. Figure 4(a)-4(c) displays three uncoated polyimide implants, while Figure 4(d)-4(f) displays three PGA coated devices. Figure 4 shows the extent of astrocyte response and their resulting conformation change, characterizing the level of glial scar formation surrounding the electrodes. The images show a minimum glial scar sheath surrounding the device, observed through the radial dark rim immediately surrounding the device. Highlighted with the arrows in Figure 4(d)-4(f), PGA coated implants show an increased amount of scar tissue on one

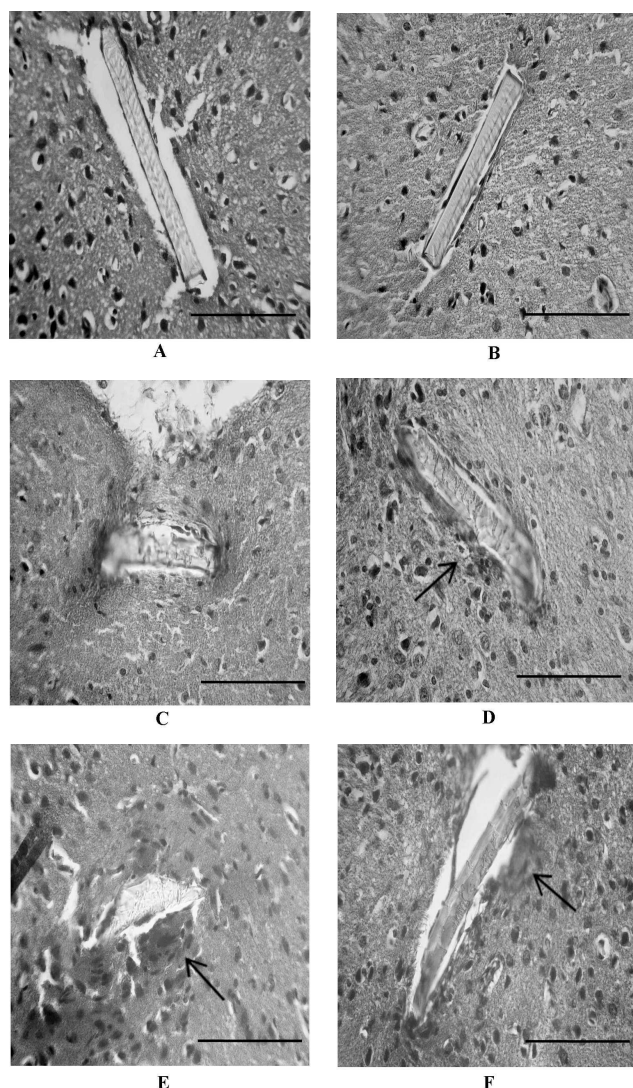


FIG. 3: H&E images of polyimide devices were taken to characterize the cell behavior in the central nervous tissue immediately surrounding the electrode device. Devices were implanted in various areas of the brain tissue. Sections were processed and stained at various depth from the implant surface to determine the cell behavior alongside the implant. The scale-bar in each image represents $100\ \mu\text{m}$. a)-c) Three uncoated polyimide devices were implanted to determine typical cell density along the device. d)-f) Three PGA coated polyimide devices were implanted to determine typical cell behavior around the device. Arrows show the increased cell density around the PGA coated polyimide electrodes.

side of the device. However, this tissue response is still minimal, remaining consistent with the previous H&E results.

Discussion

Despite the extensive work in neural devices, the need for better mechanical and biological compatible designs still exists. A further understanding of polyimide and

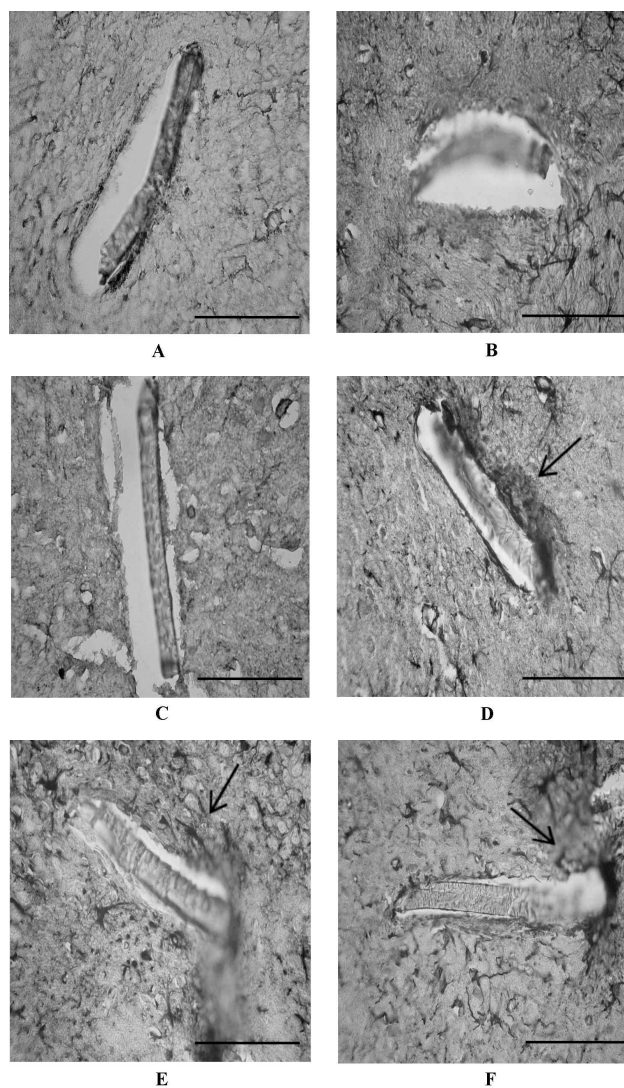


FIG. 4: GFAP immunocytochemistry images of polyimide devices were taken to characterize the tissue immune response. Devices were implanted in various areas of the brain tissue. Sections were processed and stained at various depth from the implant surface to determine the extent of glial scarring. The scale bar in each image represents $100\ \mu\text{m}$. a)-c) Three uncoated polyimide devices were implanted to assess the immune response. d)-f) Three PGA coated polyimide devices were implanted to determine the tissue response. Arrows show the increased scar tissue around the PGA coated polyimide devices.

PGA determines their applicability to implantable electrodes. In chronic implants, a device must be accepted by the surrounding tissue with a minimal foreign body response, reduce the relative mechanical strain while being rigid enough for surgical insertion, and provide long-term electrical functionality. To mechanically enhance the structural stiffness of the electrodes, devices were melt-coated with PGA. In this comparison investigation we demonstrate the biocompatibility of polyimide devices with and without enhanced mechanical rigidity.

Mechanical properties play a key role of implantable designs when devices need to be inserted through the brain tissue, utilizing PGA aids to overcome this challenge. The glass transition temperature of PGA allows it to start softening once implanted into the tissue, yet it's stable at room temperatures. Our results have shown that PGA causes little change in the pH of the surrounding tissue while it degrades; in vitro results have shown that PGA degrades rapidly within days. Changing the saline solution daily in our experiment represents the continuous fluidic exchange present in physiological processes. PGA provides the temporary mechanical rigidity required for surgical insertion of polyimide devices, while causing minimal change to the surrounding physiological environment during degradation. Histology experiments characterize the tissue response of both uncoated and PGA coated polyimide devices.

The histology results appeared to show the brain tissue lacking to adhere to the implanted device. However, it has been previously documented that cells adhere to polyimide material during in vitro studies.⁴ One plausible cause for the lack of cell adhesion in our investigation stems from the tissue preparation and fixation procedures used. Preceding paraffin embedding, the tissue was processed through a series of alcohol dehydration steps. This dehydration caused the tissue to collapse inwards towards itself, and in turn the tissue began to pull away from any outside boundaries including those of the implanted device. Therefore, the tissue appeared not to adhere to the polyimide in some H&E and immunochemistry images.

The polyimide device proves to be successfully biocompatible in central nervous system tissue, as shown by the histology results documented here. The H&E images demonstrate a minimal foreign body response and reduced tissue reaction. The cells do not encapsulate or isolate the polyimide device, allowing the electrode to maintain electrical recording functionality. In devices with PGA coating, a one-sided cell-material interaction response was observed for both H&E and immunochemistry results, suggesting that PGA may have cause a more severe tissue response relative to polyimide. The reaction seen with PGA is not extensive enough to inhibit the electrical functionality of the device.

In addition to the H&E results, the immunochemistry results show a minimum tissue immune response to the implanted devices. The GFAP immunochemistry images display the extent of glial scar formation around the device. An accumulation of scar tissue from an immune

response would isolate the device from central nervous system tissue, therefore disabling the functional capability of the device. However our results show the lack of an extreme immune response developed from the long-term implants with the absence of an encapsulating excessive radial sheath of scar tissue surrounding the device. Ideally, the astrocytes would exhibit no conformation change around the site of implantation. Our results show only a slight immune response to the polyimide devices, with a thin glial scar tissue sheath. Consistent with the H&E results, immunochemistry images show a one-sided tissue response with PGA coated devices. The glial scarring around the device suggests a mere material response to the implant.

Conclusion

Compared to previous electrode materials such as tungsten microwire and silicon, polyimide has proven to make the optimal material choice for neural device designs with chronic applications. Polyimide's mechanical flexibility provides it capability to significantly decrease the relative micromotion strain in the surrounding central nervous system tissue. To temporarily enhance the structural rigidity of the device for surgical insertion purposes, PGA is melt-coated onto the electrode interface. In this study we have demonstrated the biocompatibility of uncoated and PGA coated polyimide implants. The material properties of polyimide have allowed the device to be accepted into the surrounding brain tissue, while also maintaining proper electrical functionality. Such insights provide valuable options for designing future neural interfaces.

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